THESIS

"LEUKOPENIA IN RATS"

And

"THE ABSORPTION AND STORAGE OF CAROTENE IN THE RAT"

by K.Woods
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April/46. U. of Alberta

U N I V E R S I T Y O F A L B E R T A

FACULTY OF ARTS AND SCIENCES

This is to certify that the undersigned have read and recommend to the Committee on Graduate Studies for acceptance, a thesis submitted by Kenneth Woods, B. Sc., entitled:

1. "Leukopenia in Rats."
2. "Absorption and Storage of Carotene in the Rat."

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LEUKOPENIA IN RATS

and

ABSORPTION AND STORAGE OF CAROTENE IN THE RAT.

A THESIS

submitted in conformity with the requirements for the degree of Master of Science by the University of Alberta.

By

Kenneth Woods.

Department of Biochemistry

University of Alberta

April, 1946.

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SECTION A

LEUKOPENIA IN RATS.

INTRODUCTION

LEUKOPENIA IN RATS

INTRODUCTION

The usefulness of some valuable therapeutic agents is restricted in some cases by the development of leukopenia (diminution in the number of leukocytes), and less frequently by granulocytopenia or agranulocytosis (decrease in number or disappearance of the granular series of leukocytes). Many agents have been implicated in these phenomena, among these aminopyrine, the sulfonamides and thiouracil are the common offenders.

The mechanism by which leukopoietic depression is produced is not clear and it has been suggested that the leukopenia is due to direct depression of bone marrow activity (1), interference in enzyme systems (2, 3) and lowered bacterial synthesis in the intestine, of a substance, which stimulates bone marrow activity (4).

Experimental leukopenia produced in albino rats fed sulfonamides in a purified diet present the following characteristics: depletion of mature granulocytes in bone marrow (5, 6), lesions of blood vessels, voluntary muscles, the heart and liver (less often) and haemorrhages into various organs and subcutaneous tissues (2, 3, 7, 8). Death occurred in the majority of cases. Calloman and his associates (20) showed deceleration and cessation of growth. The reviewer in (21) observed that terminal shock manifested itself as apathy, diminution in body temperature, flaccididy of skin and muscle. A United States Public Health report (22) shows a failure of cut veins to bleed and increased viscosity of the blood.

Various therapies have been effective in correcting experimental leukopenia and granulocytopenia. Axelrod et al. (9) and Spicer et al. (11) treated this condition successfully using liver and liver extracts. Daft and his associates (10), using sulfaguanidine and sulfasuxidine, showed the effectiveness of feeding crystalline folic acid. Goldsmith et al. (12) successfully treated neutrophilic granulocytopenia induced by thiouracil (fed to rats) with solubilized liver. Waisman and Elvehjem (13) suggested that folic acid was the active agent in this liver fraction. Daft and Sebrell (10) provide experimental evidence for this view. In a critical review (4) it is pointed out that the effect of folic acid may be indirect, in that folic acid is required by the coliform bacteria in the intestine for the production of some accessory substance which in turn produces the granulocytic response. The effectiveness of pyridoxine in elevating the leukocyte count in the anaemia of pellagra (29) prompted Cantor and Scott (14) to use this agent clinically. Their results led them to conclude that pyridoxine acts by direct stimulation of the myelocytic elements of the bone marrow.

It was to test the validity of this conclusion that the following investigations were planned.

Deficiency symptoms of pyridoxine common to rats, dogs, pigs and chicks are lack of growth, anaemia and convulsions (15). Not all investigators have noted convulsions. Acrodynia has been observed only in rats. None noted that pyridoxine affected male rats more than females. Neither Hegsted and his

associates (15) nor Kornberg et al. (16) observed leukopenia in pyridoxine deficient rats. Bethall et al. (1) and Fouts et al. doubt the importance of pyridoxine in erythropoiesis in the rat. They report that only moderate anaemia and leukopenia were produced by pyridoxine deficiency in albino rats. Chick, MacRae and Martin (18) found only a slight reduction in haemoglobin in pyridoxine deficient rats.

PART I

"The effect of thiouracil and sulfaguanidine on the white cell count in pyridoxine deficient rats."

PLAN OF EXPERIMENT

To show the effect of thiouracil and sulfaguanidine on the white cell count in piebald rats.

Animals

Three groups of piebald rats from 6 to 8 weeks of age were used in each experiment. Each group of 6, consisting of an equal number of males and females, was placed in a separate cage and fed a B-complex free diet to which thiouracil, etc. was added. They were allowed water and the diet ad lib.

The purified diet consisted of:

Sucrose	68%
Vitamin free casein	18%
Vegetable oil	8%
U. S. P. cod liver oil	2%
U. S. P. salt mixture	4%

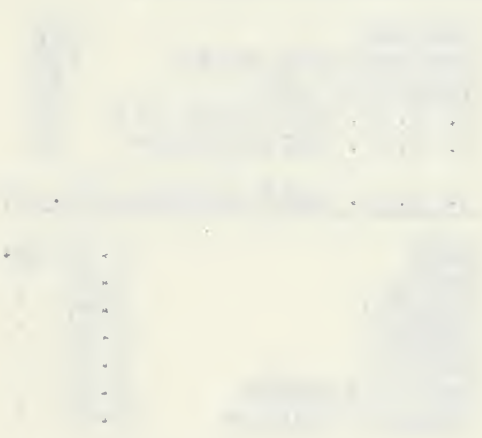
U. S. P. salt mixture (No. 2)

NaCl	1.73 gm.
$MgSO_4$	5.45 "
NaH_2PO_4	3.47 "
KH_2PO_4	9.54 "
$CaHPO_4$	5.40 "
Ferric citrate	1.18 "
Calcium lactate	13.00 "

The animals were weighed once every three days.

During the entire experimental period a protocol was kept and remarks made on evidence of nutritional deficiency, date of death and any gross changes in urine and faeces.

When the animals showed the first outward signs of dietary defects, they were fed the daily supplement of pyridoxine free B-complex factors. Graying and loss of hair were usually the first indications of pyridoxine deficiency.



Counting

A total and a differential white cell count were performed on each animal every two weeks using the common clinical apparatus and procedure.

(a) Total white cell count

Procedure:

The tip of each tail was nicked with scissors and the tail milked to stimulate the free flow of blood. The sample was taken after the first two drops had been discarded. This was diluted with acetic acid, the pipette shaken vertically for 30 seconds and horizontally for 10 seconds, and then allowed to stand for one half hour. The first two drops from the pipette were discarded and the counting chamber carefully filled under the cover slip.

Using the low power (8X) of the microscope with the diaphragm almost closed and the condenser up, the total number of leukocytes in the four corner squares was counted in the usual manner. This total multiplied by 50 gives the total number of leukocytes in one cubic millimetre of blood (1).

(b) Differential white cell count

The differential count was performed on a thin smear stained with Leichman stain.

Blood smears were made from drops of freely flowing blood. Care was taken to obtain thin, evenly spread smears. The dried smear was flooded with Leichman's stain for three minutes, then buffer added, mixed by blowing, and allowed to stand five minutes. It was then washed with distilled water

Variation

A total and a differential white cell count were performed on each animal every two weeks using the common clinical procedure and technique.

a) Total white cell count

Procedure:

The tip of each tail was nicked with scissors and a small amount of blood collected in the pipette of the counting chamber. The pipette was then filled with Hayems solution, the fingers rolled vertically for 30 seconds and horizontally for 30 seconds, and then allowed to stand for one half hour. The first two drops from the pipette were discarded and the counting chamber carefully filled under the cover slip.

Using the low power (XP) of the microscope with the diaphragm almost closed and the sub-stage lowered so, the total number of leucocytes in the four corner squares was counted in the usual manner. This total multiplied by 50 (i.e. the dilution of leucocytes in the white pipette) gives the total number of leucocytes in one cubic millimetre of blood (1).

(2) Differential white cell count

The differential count was performed in a like manner, identical with Leishman stain.

Slight changes were made from time to time in the technique. Thus was taken to obtain thin, evenly spread smears. The stain needed was flooded with Leishman's stain for three minutes. Then buffer added, rocked by slanting, for a further three minutes. It was then washed with distilled water

until the excess stain was removed. The end of each dried slide was labelled with a strip of gummed paper which recorded the animal, its group and the date.

Using the oil immersion objective and a drop of cedar oil, a differential count was made on 100 stained leukocytes. The count was differentiated as follows:

(1) per cent Polymorphs
Neutrophils
Eosinophils
Basophils

(2) per cent Lymphocytes

(3) per cent Monocytes.

Where a field could not be brought clearly into focus or doubt remained in differentiation, another count was made.

RESULTS

Shown in the tables and graphs. B-complex free diet plus a drug, supplemented by B-complex factors less pyridoxine.

(1) Tables and Graphs I and II.

Control group.

(2) Tables and Graphs III and IV.

One per cent thiouracil in diet.

(3) Tables and Graphs V and VI.

One per cent sulfaguanidine in diet.

Pyridoxine Deficient Diet.

Table I. Total leukocyte count per cubic millimetre blood

Rat	Weeks after experiment began											
	0	1	2	3	4	5	6	7	8	9	11	13
o-m	9700	9000	16450	13500	11800	6050	7450	6650	4850	4800	7100	--
o-f	6800	7350	14900	13200	10500	9100	8600	5950	5600	5650	7450	6050
R-m	7200	10250	17000	11700	16000	9750	8500	8800	6500	6250	8950	6950
R-f	6900	8900	12650	15900	14800	7300	7100	6200	5400	8100	6600	7000
L-m	7000	7600	15150	8550	16150	6350	4750	3250	3200	5800	6350	--
L-f	8000	12100	15900	16900	13400	6200	4850	5200	11000	6600	16200	9100

m = male
f = female

o = no marked ear
R = right ear marked
L = left ear marked

Table II. Differential count per cubic millimetre blood

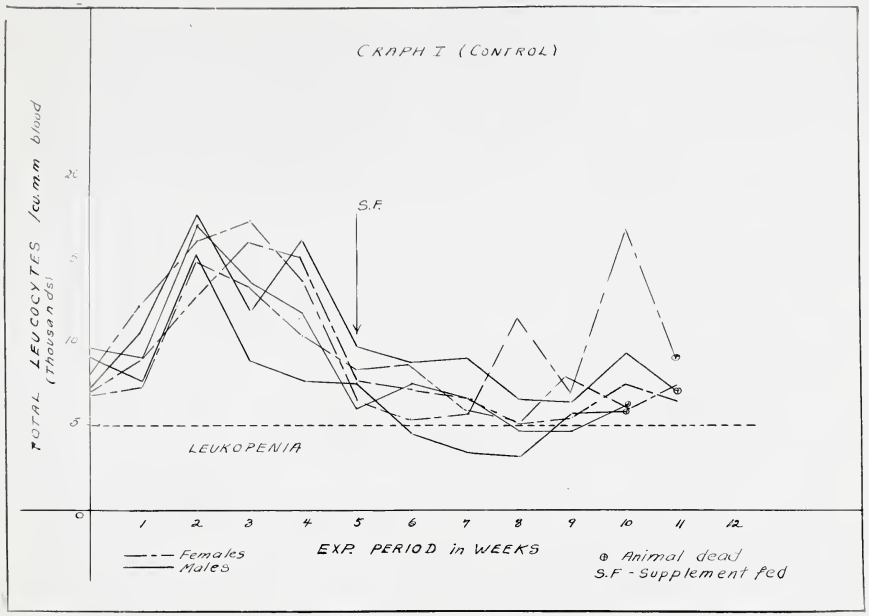
Rat		Weeks after experiment began											
		0	1	2	3	4	5	6	7	8	9	11	13
o-m	P	1455	1980	1974	3510	4130	1996	1564	1662	1697	1440	3124	--
	L	8148	7020	14476	9890	7670	3993	5885	4921	3104	2312	3976	--
	M	97	0	0	0	0	60	0	66	49	48	0	--
o-f	P	680	1543	1937	3300	4725	2093	2064	1250	1008	6780	2458	1573
	L	6052	5733	12963	9900	5775	7007	6536	4581	4536	4915	4991	4477
	M	68	74	0	0	0	0	0	119	56	56	0	60
R-m	P	792	1025	1700	3510	5600	1852	1615	3960	1755	1562	1422	1598
	L	6408	9225	15300	8190	10400	7800	6800	4840	4745	4687	7607	5351
	M	0	0	0	0	0	97	85	0	0	0	0	0
R-f	P	690	1691	1518	5565	4884	3431	1704	1364	1836	4212	2904	3430
	L	6210	7120	11132	10335	9768	3869	5325	4836	3510	3888	3696	3920
	M	0	89	0	0	148	0	71	0	54	0	0	0
L-m	P	980	1672	1818	1924	6460	2413	902	4455	800	1508	1651	--
	L	7550	5852	13332	6412	9690	3873	3848	2790	2400	4234	4699	--
	M	70	76	0	0	0	63	0	0	0	58	0	--
L-f	P	960	1815	2226	2535	2010	1860	970	1196	3300	2442	7776	5096
	L	7040	10160	13674	14365	11256	4340	3880	4004	7610	4158	8424	4004
	M	68	121	0	0	134	0	0	0	90	0	0	0

o = no marked ear
R = right ear marked
L = left ear marked

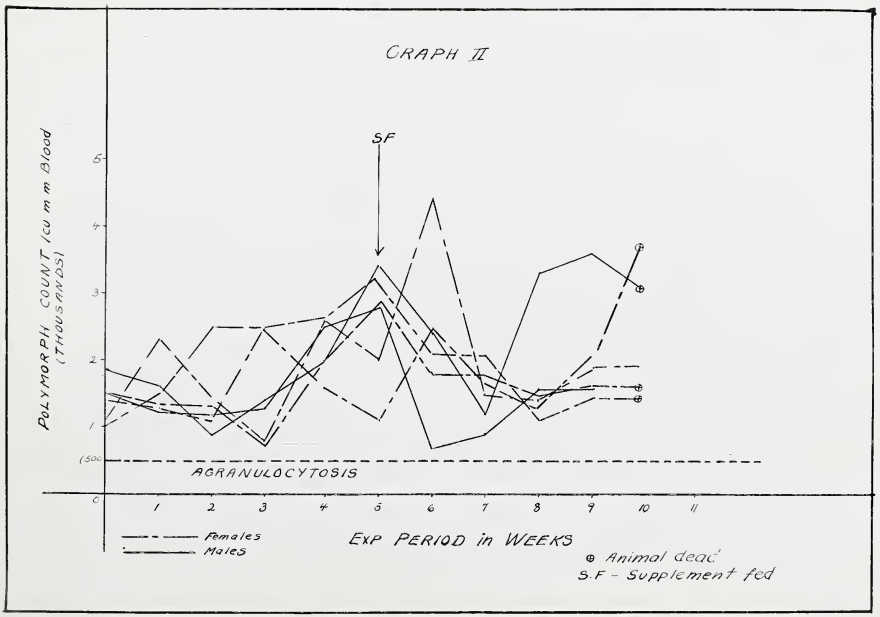
m = male
f = female

P = Polymorphs
L = Lymphocytes
M = Monocytes

Graph I. Effect of a pyridoxine deficient diet on the total leukocyte count.



Graph II. Effect of a pyridoxine deficient diet on the granulocytic (polymorph) count.



Pyridoxine Deficient Diet + 1% Thiouracil.

Table III. Total leukocyte count per cubic millimetre blood

Rat	Weeks after experiment began										
	0	1	2	3	4	5	6	7	8	9	11
o-m	7500	6250	6800	6800	6800	8800	5800	6400	6350	8950	--
o-f	6050	8800	6850	6350	6800	8450	9000	9000	6250	7800	6000
R-m	6250	11700	6800	7400	8000	11000	9600	6150	6700	10600	10200
R-f	5100	13700	8650	7650	9000	11800	6600	6400	5500	7800	7200
L-m	5800	9950	14050	12800	9800	10050	13000	5050	6550	9400	--
L-f	6250	8100	6000	6000	6000	6250	6200	6400	3500	4500	3400

m = male
f = female

o = no marked ear
R = right ear marked
L = left ear marked

Pyridoxine Deficient Diet + 1% Thiouracil.

Table IV. Differential count per cubic millimetre blood

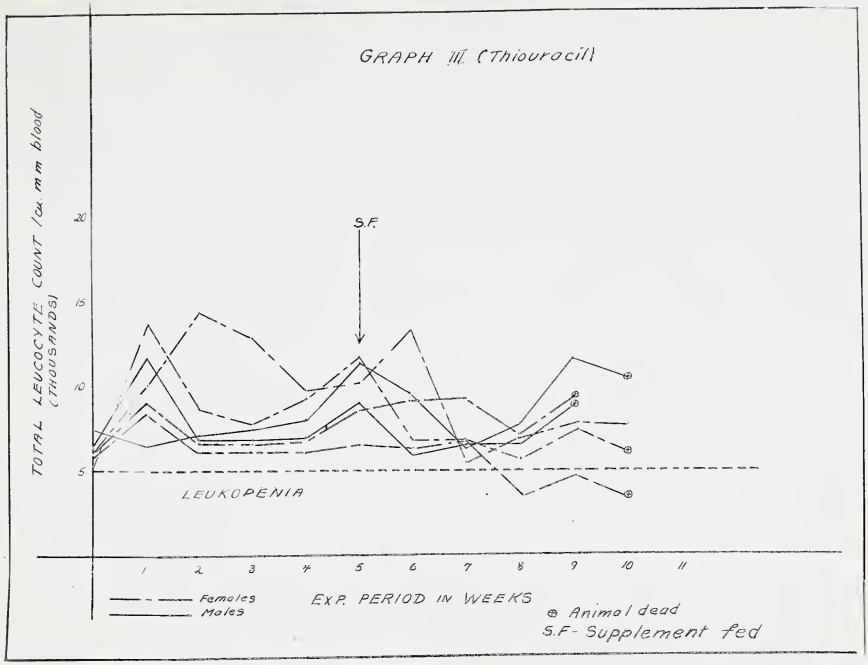
Rat		Weeks after experiment began										
		0	1	2	3	4	5	6	7	8	9	11
o-m	P	1500	1240	1020	1360	2516	2816	754	960	1587	1521	--
	L	5850	4898	5780	5440	4284	5984	5046	5376	4725	7428	--
	M	150	62	0	0	0	0	0	64	0	89	--
o-f	P	1500	1408	1370	7620	2040	2856	1800	1800	1444	1872	3780
	L	4500	7392	5372	5544	4692	5492	7200	7110	11836	5850	2220
	M	0	88	68	0	68	84	0	90	0	78	0
R-m	P	1860	1872	884	1480	2080	3410	2400	1230	3283	3604	2856
	L	4278	9711	5916	5772	5840	7480	7200	6919	3417	6996	7344
	M	62	117	0	148	80	110	0	61	0	0	0
R-f	P	1871	2329	1470	851	2520	3422	2112	2048	1100	1404	1440
	L	4029	10960	7179	6808	6390	8378	4488	4352	4400	5616	7088
	M	0	411	0	0	90	0	0	0	0	0	72
L-f	P	1566	1584	2660	2560	2548	2000	4420	1464	1375	1598	--
	L	4176	8316	11340	10240	7252	8000	8580	3535	5070	7802	--
	M	58	0	0	0	0	0	0	50	65	0	--
L.R-f	P	1488	1377	1140	1200	1680	1062	2542	1600	700	1575	1632
	L	4464	6723	4860	4800	4320	5188	3658	4800	800	2925	1768
	M	248	0	0	0	0	0	0	0	0	0	0

o = no marked ear
R = right ear marked
L = left ear marked
L.R = both ears marked

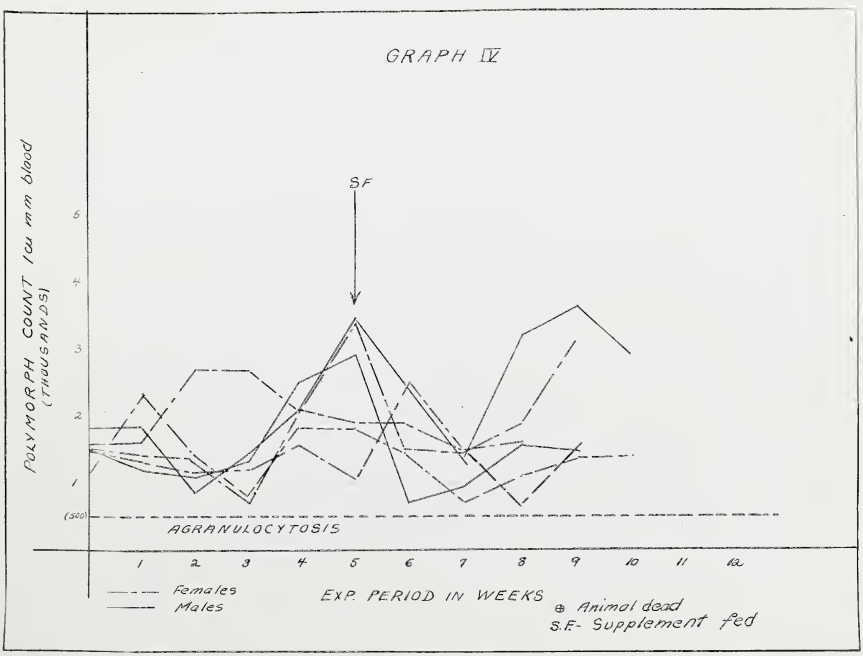
m = male
f = female

P = Polymorphs
L = Lymphocytes
M = Monocytes

Graph III. The effect of a pyridoxine deficient diet + 1% thiouracil on the total leukocyte count.



Graph IV. The effect of a pyridoxine deficient diet + 1% thiouracil on the granulocytic count.



Pyridoxine Deficient Diet + 1% Sulfaguanidine.

Table V. Total leukocyte count per cubic millimetre blood

Rat	Weeks after experiment began											
	0	1	2	3	4	5	6	7	8	9	11	12
o-m	6200	10500	9350	12000	6350	4900	4450	5800	3350	5700	--	--
o-f	6600	17500	9600	10900	5550	5400	5700	4200	3250	5600	--	--
R-m	6700	9850	9400	10550	5100	5100	4400	5900	3950	6000	--	--
R-f	12500	20500	13050	17000	12500	12400	12450	10700	4650	6150	4900	2800
L-m	6600	12000	12400	13600	5500	5600	6800	5850	3200	4400	6550	--
L-f	5400	16400	13150	11400	6050	6400	6400	6450	3200	5650	6500	6400

m = male
f = female

o = no marked ear
R = right ear marked
L = left ear marked

Pyridoxine Deficient Diet + 1% Sulfaguanidine.

Table VI. Differential Count
per cubic millimetre blood

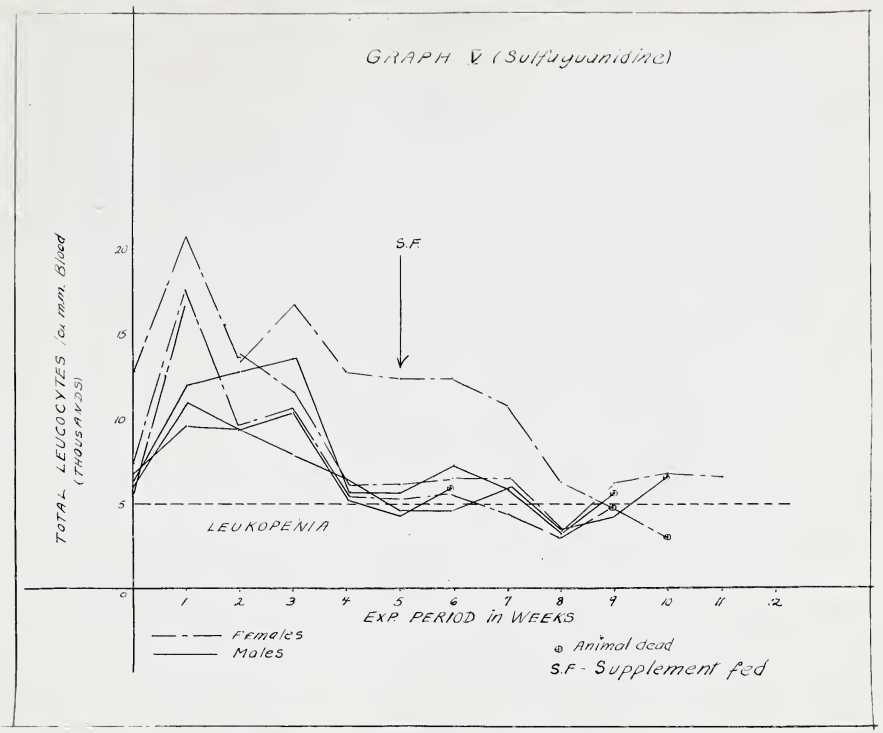
Rat		Weeks after experiment began. 0	1	2	3	4	5	6	7	8	9	11	12
o-m	P	1240	1680	1122	1200	2476	2476	1421	222	1392	1653		
	L	4744	8820	8134	10800	3873	3873	3479	4227	4408	3990		
	M	186	0	93	0	0	0	0	0	0	57		
o-f	P	1122	3150	1824	1308	1170	1100	1296	1710	756	1792		
	L	5412	14350	7776	9592	4440	4400	4050	3990	5133	3808		
	M	66	0	0	0	0	0	54	0	0	0		
R-m	P	1742	1764	1222	2730	1836	1071	1071	352	767	1980		
	L	4824	8036	8176	7770	3213	3213	4029	4048	5120	4020		
	M	134	0	0	0	51	51	0	0	0	0		
R-f	P	2500	2255	3640	6800	9500	9500	2356	5580	3103	1537	686	448
	L	9875	18245	9360	10200	3000	3000	10044	6820	7490	4514	4214	2352
	M	125	0	130	0	0	0	0	0	107	61	0	0
L-m	P	1650	2280	1488	3672	2664	2664	1176	1360	1111	528	4810	
	L	4752	9600	10912	9928	9928	2886	4424	5440	4738	3828	1690	
	M	198	120	0	0	0	0	0	0	0	44	0	
L-f	P	1080	2296	1834	1824	1824	2843	1280	1152	903	1582	2600	512
	L	4326	14104	11266	9576	9576	3206	5120	5248	5547	4068	3900	5888
	M	0	0	0	0	0	0	0	0	0	0	0	0

o = no marked ear
R = right ear marked
L = left ear marked

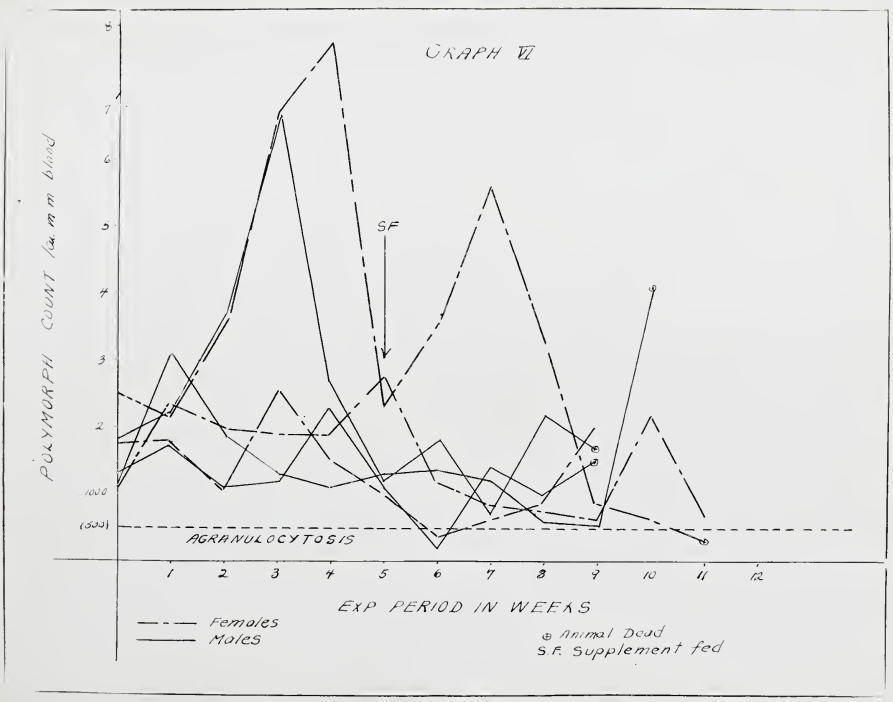
m = male
f = female

P = Polymorphs
L = Lymphocytes
M = Monocytes

Graph V. The effect of a pyridoxine deficient diet plus 1 % sulfaguanidine on the total leukocyte count.



Graph VI. The effect of a pyridoxine deficient diet plus 1 % sulfaguanidine on the granulocytic count.



DISCUSSION

When the diet was deficient in pyridoxine the leukocytes in nearly all animals followed the same trend (Table I, Graph I). There was an initial rise in the total leukocytes, followed by a steady decline to leukopenia in two animals. After feeding the pyridoxine free B-factors there was no evidence of leukopoiesis. This may be attributed to pyridoxine deficiency. Agranulocytosis was not produced (Table II and Graph II). Almost all animals died before a low enough count could be obtained. Animals approaching death showed symptoms typical of pyridoxine deficiency (loss of weight, stiffness, body tremors). Tables III and IV illustrate the results of feeding 2 per cent thiouracil in the pyridoxine deficiency diet. The initial rise in leukocyte count is not as great as in the case of simple pyridoxine deficiency. Thiouracil evidently tended to depress the white cell count more quickly than was the case in the B-free diet. A less even trend of counts may be attributed to the different sensitivity of individual animals to the drug. Leukopenia was not produced to any severe degree and agranulocytosis not at all. The thiouracil in the pyridoxine deficient ration did not lower the cell count below that of the first experiment. Animals approaching death showed symptoms similar to those described in the first experiment.

A severe degree of leukopenia was produced using one per cent sulfaguanidine in the diet (Table V and Graph V). Nearly all animals showed a low total white cell count. Agranulocytosis developed in two animals. There is evidence of greater

leukocyte depression in this group than in either of the previous experiments. The very low counts might be attributed to unusual sensitivity toward sulfaguanidine.

SUMMARY

(1) A moderate degree of leukopenia was produced in rats on a pyridoxine deficient diet.

(2) A similar degree of leukopenia was produced using ½ per cent thiouracil in the diet.

(3) A greater degree of leukopenia was produced in rats on a pyridoxine deficient diet plus one per cent sulfaguanidine.

(4) Animals only on the sulfaguanidine diet showed agranulocytosis.

(5) Nearly all animals showed the characteristic symptoms typical of those on a pyridoxine deficient diet (15, 17, 26).

PART II

"The effect of a pyridoxine supplement on the leukocyte count in rats fed a pyridoxine free diet plus gardan, pyramidon and sulfaguanidine."

PLAN OF EXPERIMENT

Animals

Animals were prepared in the manner already described. The pyridoxine supplement was administered by subcutaneous injection when leukopenia became evident. Periodicity of injection and the results obtained are indicated in tables and graphs following.

Counting

As in Part I.

RESULTS

Indicated in the tables and graphs. B-complex free diet plus a drug, supplemented by B-complex factors less pyridoxine, and separately supplemented by pyridoxine subcutaneously.

(1) Tables and Graphs VII and VIII.
.25 per cent gardan in the diet. (Piebald rats).

(2) Tables and Graphs IX and X.
.25 per cent gardan in the diet. (Albino rats).

(3) Tables and Graphs XI and XII.
.25 per cent pyramidon in the diet. (Piebald rats).

(4) Tables and Graphs XIII and XIV.
.25 per cent pyramidon in the diet. (Albino rats).

(5) Tables and Graphs XV and XVI.
2.0 per cent sulfaguanidine in the diet. (Albino rats).

Pyridoxine + .25% Gardan.

Table VII. Total leukocyte count per cubic millimetre blood

Rat	Weeks after experiment began										
	0	2	4	7	9	11	13	15	17	19	21
o-m	18200	--	--	--	--	--	--	--	--	--	--
o-f	10240	28850	14900	8400	8300	5900	10550	25500	--	--	--
R-m	9700	17000	10150	7100	12300	9750	7000	11200	15150	14550	8500
R-f	14850	--	--	--	--	--	--	--	--	--	--
L-m	10300	12550	15900	6200	10100	5850	9050	15450	10600	--	--
L-f	12350	14400	14300	10300	13600	9950	4850	14000	11600	8900	8400

m = male
f = female

o = no marked ear
R = right ear marked
L = left ear marked

Pyridoxine + .25% Gardan.

Table VIII. Differential count per cubic millimetre blood

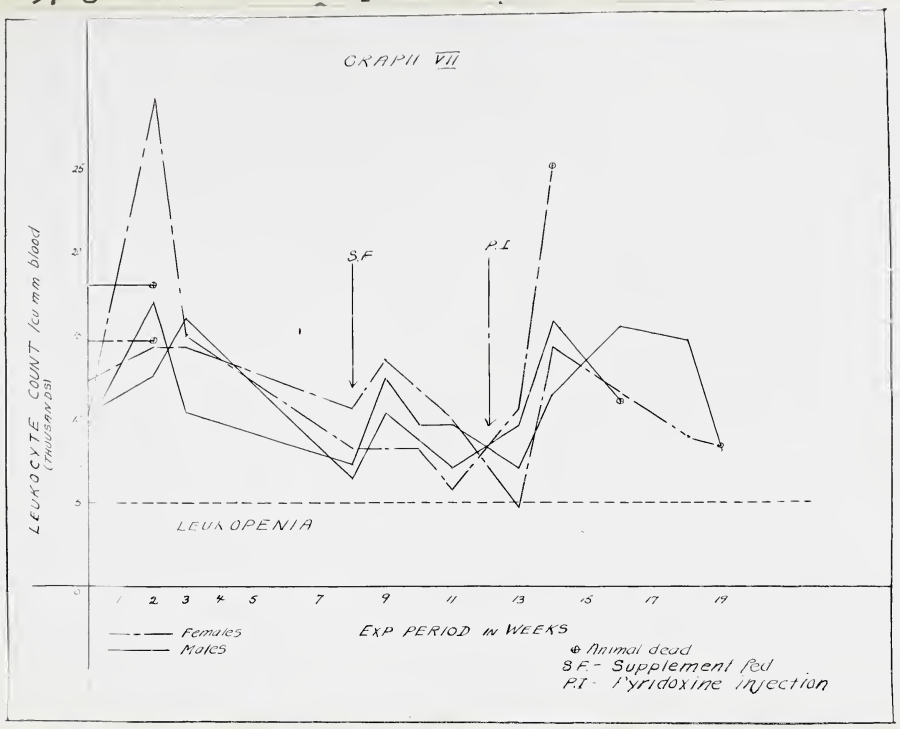
Rat		Weeks after experiment began										
		0	2	4	7	9	11	13	15	17	19	21
o-m	P	7826	--	--	--	--	--	--	--	--	--	--
	L	10374	--	--	--	--	--	--	--	--	--	--
	M	0	--	--	--	--	--	--	--	--	--	--
o-f	P	1428	2885	2384	3444	2407	1003	2215	--	--	--	--
	L	8670	25920	12516	4956	5893	4897	8295	--	--	--	--
	M	102	0	0	0	0	0	0	--	--	--	--
R-m	P	1358	6460	2323	2769	7626	4095	2240	3360	6211	1700	--
	L	8342	10540	7777	4331	4674	5655	4760	7728	8938	6800	--
	M	0	0	0	0	0	0	0	112	151	0	--
R-f	P	2970	--	--	--	--	--	--	--	--	--	--
	L	11731	--	--	--	--	--	--	--	--	--	--
	M	148	--	--	--	--	--	--	--	--	--	--
L-m	P	1751	5750	8904	2480	6565	1462	1176	2163	7844	--	--
	L	8343	6750	6996	3720	3535	4387	7873	13287	2756	--	--
	M	0	0	0	0	0	0	0	0	0	--	--
L-f	P	1845	3888	2145	3605	6256	2288	1747	7140	3132	2581	2100
	L	10332	10512	12155	6695	7344	7661	3152	6860	8468	6319	6300
	M	123	0	0	0	0	0	0	0	0	0	0

o = no marked ear
R = right ear marked
L = left ear marked

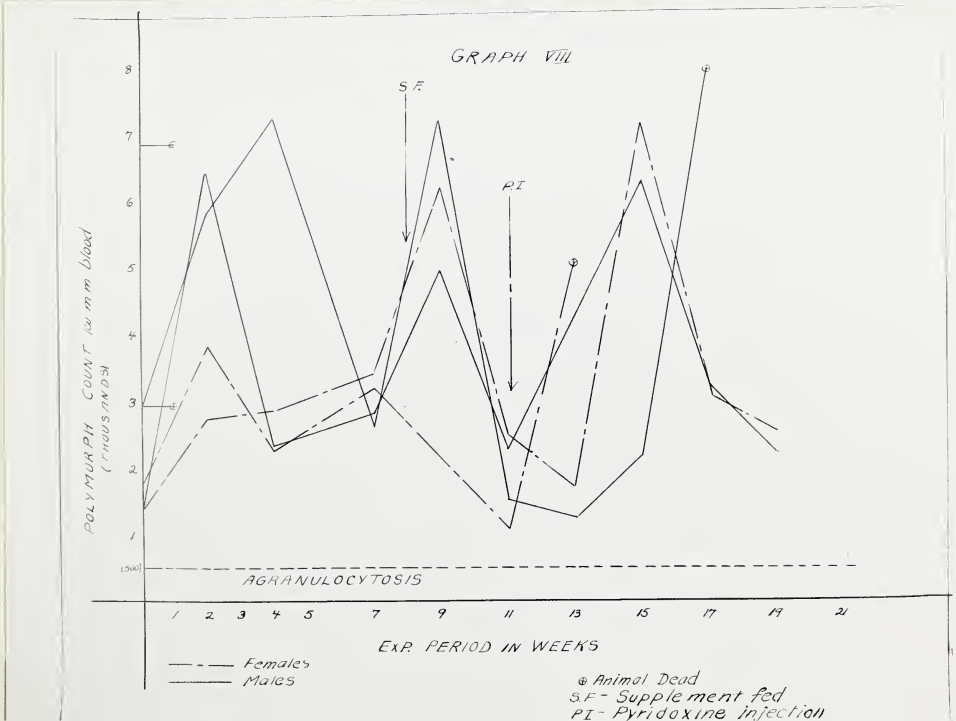
m = male
f = female

P = Polymorphs
L = Lymphocytes
M = Monocytes

Graph VII. The effect of pyridoxine on leukopenia induced by .25% gardan in a B-complex free diet (Piebald rats,



Graph VIII. The effect of pyridoxine on a low polymorph count induced by .25% gardan.



Pyridoxine + .25% Gardan.

Table IX. Total leukocyte count per cubic millimetre blood

Rat	Weeks after experiment began										
	0	2	5	7	9	11	13	15	17	19	20
o-m	8100	13800	15300	10500	9050	15550	12300	23200	21200	--	--
o-f	9000	17250	15400	23400	8550	15800	8050	19500	16700	12500	--
R-m	8950	12650	5300	11750	8500	15050	12200	20600	18400	14800	500
R-f	8500	21500	17300	17500	8450	13250	7400	19200	8600	15300	--
L.R-f	7200	14400	14550	12000	5700	11100	12450	22750	8100	--	--
L-f	6850	18700	16900	17200	8850	17350	7850	14300	10050	16900	--

m = male
f = female

o = no marked ear
R = right ear marked
L = left ear marked
L.R = both ears marked

Pyridoxine + .25% Gardan.

Table X. Differential count per cubic millimetre blood

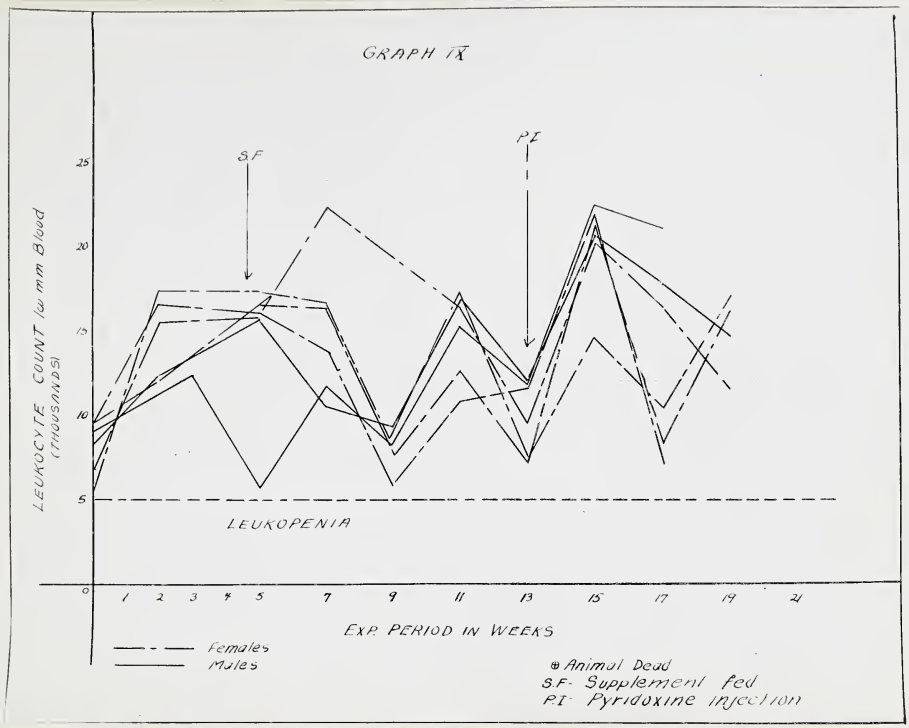
Rat		Weeks after experiment began										
		0	2	5	7	9	11	13	15	17	19	20
o-m	P	1377	1932	3360	1890	3420	5270	3444	5800	6360	--	--
	L	6723	11868	11934	8610	5580	10230	8856	17400	14840	--	--
	M	0	0	0	0	0	0	0	0	0	--	--
o-f	P	1350	3268	3388	6786	3400	6320	1680	4095	1670	5250	--
	L	7560	13760	12012	16380	5100	9480	6320	15405	15030	7250	--
	M	70	172	0	234	0	0	0	0	0	0	--
R-m	P	1432	2394	742	5616	5610	6300	2928	4944	3680	5180	1050
	L	7518	10206	4558	6084	2890	8700	9272	15656	14720	9324	3950
	M	0	0	0	0	0	0	0	0	0	148	0
R-f	P	1275	1720	4671	4650	4200	1716	1110	2496	1806	5202	--
	L	7225	19780	12629	12950	4200	11484	6290	16704	6794	10098	--
	M	0	0	0	0	0	0	0	0	0	0	--
L.R-f	P	1440	2880	2900	6120	2280	1332	2976	3405	1620	--	--
	L	5760	11520	11600	5880	3420	9768	9300	19295	6480	--	--
	M	0	0	0	0	0	0	0	0	0	--	--
L-f	P	952	2057	2535	2408	2288	3806	468	2002	2400	6760	--
	L	5848	16456	14365	14792	6512	13494	7332	12298	7600	9971	--
	M	0	0	0	0	0	0	0	0	0	169	--

o = no marked ear
R = right ear marked
L = left ear marked
L.R = both ears marked

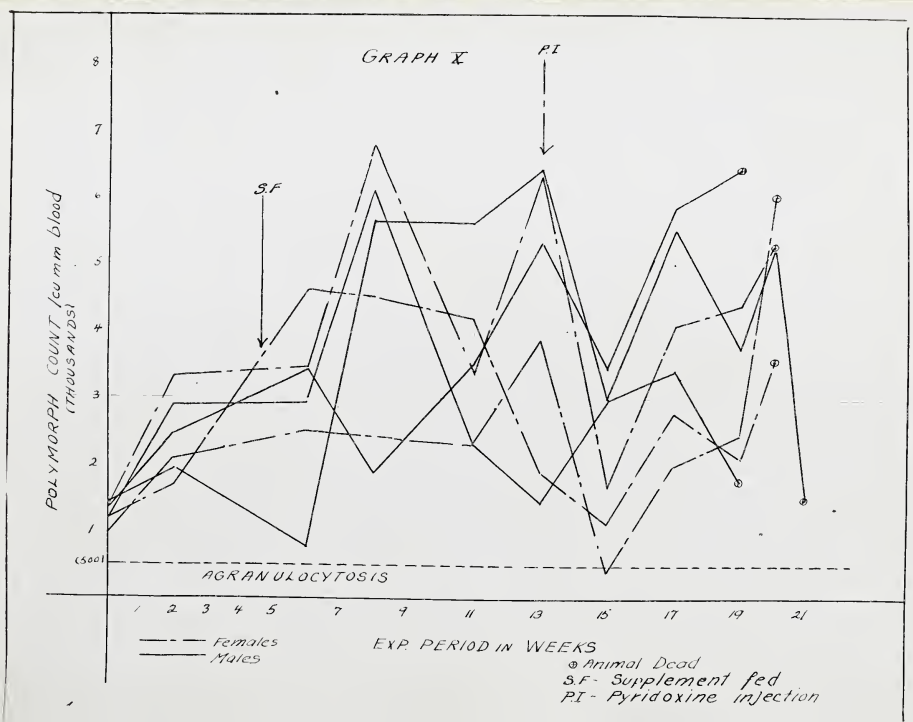
m = male
f = female

P = Polymorphs
L = Lymphocytes
M = Monocytes

Graph IX. The effect of pyridoxine on leukopenia induced by .25% gardan in a B-complex free diet (Albino rats, 7 weeks age).



Graph X. The effect of pyridoxine on a low polymorph count induced by .25% gardan.



Pyridoxine + .25% Pyramidon.

Table XI. Total leukocyte count per cubic millimetre blood

Rat	Weeks after experiment began											
	0	2	4	7	9	11	13	14	15	16	18	19
o-m	12350	6150	9800	10050	12900	7500	4100	--	--	--	--	--
o-f	12700	13050	13900	7850	13950	9850	--	--	--	--	--	--
R-m	17950	9100	14300	8600	8700	8950	8050	11200	10630	16450	14400	40000
R-f	14100	--	--	--	--	--	--	--	--	--	--	--
L-m	10000	--	--	--	--	--	--	--	--	--	--	--
L-f	10750	6100	13600	7350	9500	8050	7500	9000	--	--	--	--

m = male
f = female

o = no marked ear
R = right ear marked
L = left ear marked

Pyridoxine + .25% Pyramidon.

Table XII. Differential count per cubic millimetre blood

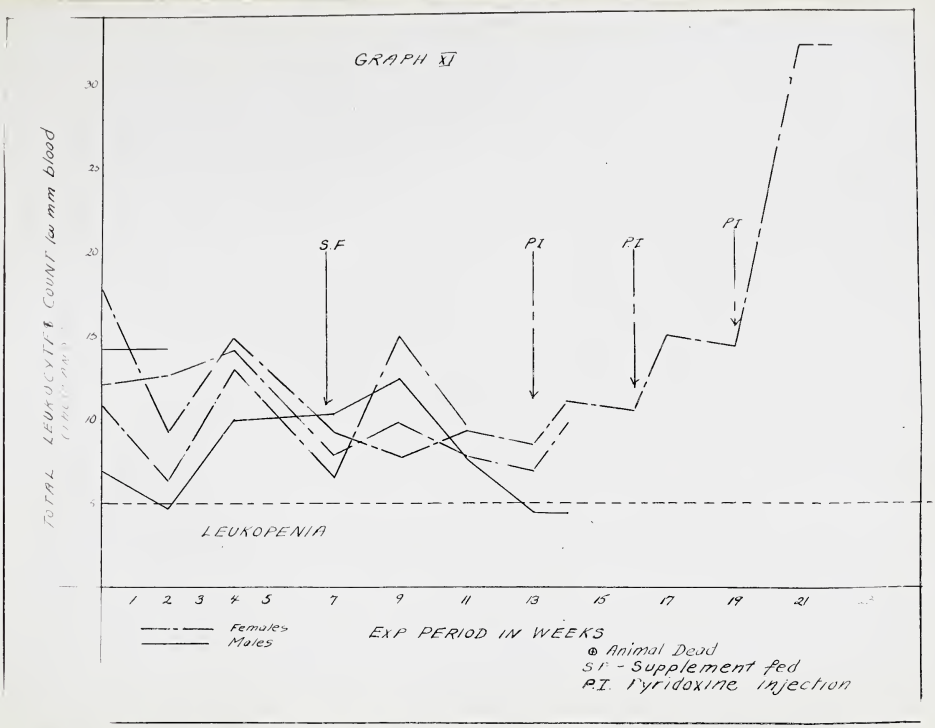
Rat		Weeks after experiment began											
		0	2	4	7	9	11	13	14	15	16	18	19
o-m	P	1476	1722	2940	5500	9159	1425	328	--	--	--	--	--
	L	10701	4428	6860	4500	3741	6075	3772	--	--	--	--	--
	M	123	0	0	0	0	0	0	--	--	--	--	--
o-f	P	1270	2600	2919	1872	3465	1666	--	--	--	--	--	--
	L	11303	10270	10981	5928	10425	8134	--	--	--	--	--	--
	M	127	130	0	0	0	0	--	--	--	--	--	--
R-m	P	7160	2275	3289	2236	2610	1611	2240	5040	2650	3608	1440	19200
	L	10740	6825	10868	6364	6090	7339	5760	6160	7950	12792	12960	28320
	M	0	0	143	0	0	0	0	0	0	0	0	480
R-f	P	4512	--	--	--	--	--	--	--	--	--	--	--
	L	9588	--	--	--	--	--	--	--	--	--	--	--
	M	0	--	--	--	--	--	--	--	--	--	--	--
L-m	P	1200	--	--	--	--	--	--	--	--	--	--	--
	L	8800	--	--	--	--	--	--	--	--	--	--	--
	M	0	--	--	--	--	--	--	--	--	--	--	--
L-f	P	1819	1464	3672	2205	5700	2817	3075	4230	--	--	--	--
	L	8881	4636	9928	5110	3800	5200	4425	4770	--	--	--	--
	M	0	0	0	0	0	0	0	0	--	--	--	--

o = no marked ear
R = right ear marked
L = left ear marked

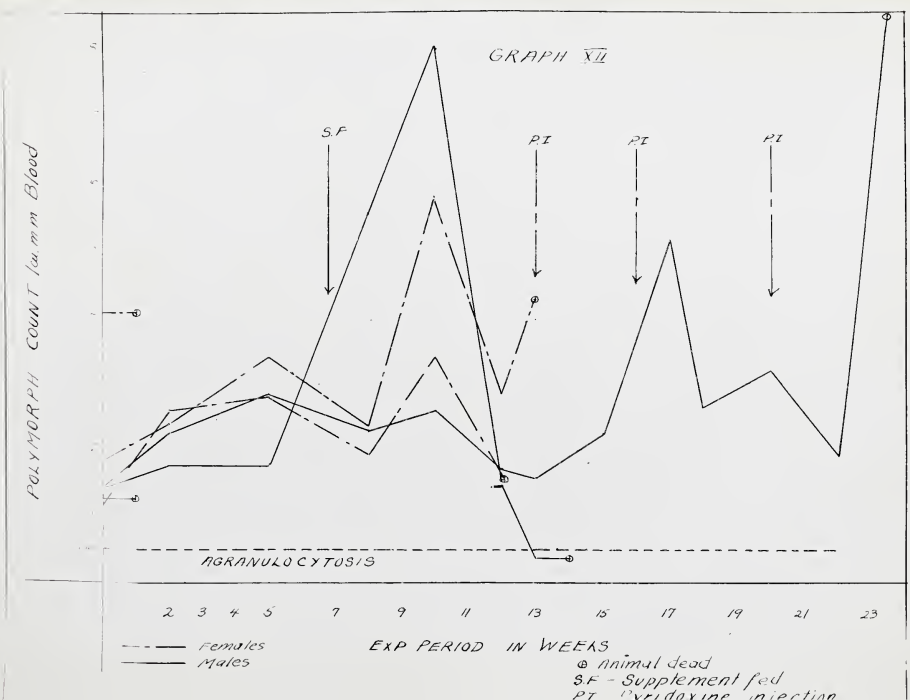
m = male
f = female

P = Polymorphs
L = Lymphocytes
M = Monocytes

Graph XI. The effect of pyridoxine on leukopenia induced by .25% pyramidon in a B-complex free diet (Piebald rats,



Graph XII. The effect of pyridoxine on a low polymorph count induced by .25% pyramidon.



Pyridoxine + .25% Pyramidon.

Table XIII. Total leukocyte count per cubic millimetre blood

Rat	Weeks after experiment began										
	0	2	5	7	9	11	13	15	18	19	20
o-m	10250	15400	10950	13300	15600	9500	17800	11150	8200	22000	12100
o-f	16100	22200	14000	14100	11350	24300	16900	16200	16400	14500	22100
R-m	12100	16200	16400	11000	10600	19950	14800	8950	8850	8800	--
R-f	10600	12950	17200	14050	11900	17100	13250	9400	12400	10700	--
L-m	12050	18750	21800	11050	8550	19700	12600	4400	9900	6800	23500
L-f	10900	13450	15100	12400	10600	11800	17400	13650	6400	21600	--

m = male
f = female

o = no marked ear
R = right ear marked
L = left ear marked

Pyridoxine + .25% Pyramidon.

Table XIV. Differential count per cubic millimetre blood

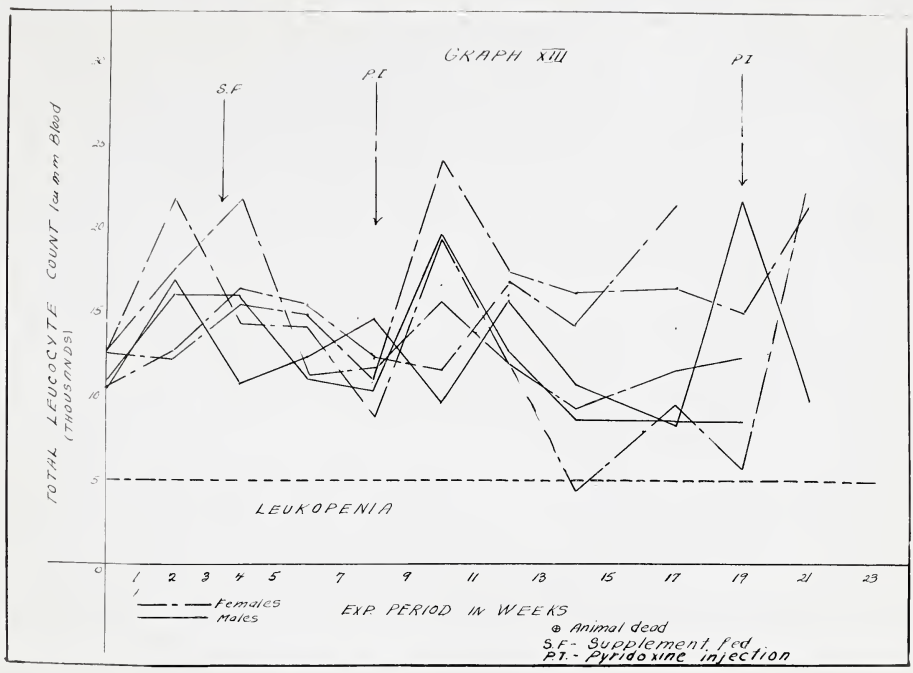
Rat		Weeks after experiment began										
		0	2	5	7	9	11	13	15	18	19	20
o-m	P	1836	3080	2180	5453	4368	2755	4628	4884	2132	6600	3630
	L	8364	12320	8720	7843	11232	6745	13172	6216	6068	1540	8470
	M	0	0	0	0	156	0	178	0	0	0	0
o-f	P	1932	1332	1960	2397	1921	11178	2535	10082	3668	2900	5746
	L	14168	20868	12040	11703	9266	13122	14366	6318	12792	11600	16133
	M	0	0	0	0	113	0	0	0	0	0	221
R-m	P	3267	4212	3936	3740	3180	6965	3108	3293	1872	2024	--
	L	8833	11988	12464	7260	7420	12935	11692	5607	7040	6776	--
	M	0	0	0	0	0	0	0	0	88	88	--
R-f	P	2226	2580	3268	4620	1666	4617	3696	3196	2728	2675	--
	L	8374	10320	13932	9380	10234	12483	9504	6204	8672	8025	--
	M	0	0	0	0	0	0	0	0	0	0	--
L-m	P	960	1683	2180	1650	1190	5910	2016	616	2376	1428	7050
	L	11040	16830	19620	9350	7310	13790	10584	3784	7524	5372	16215
	M	0	187	0	0	0	0	0	0	0	0	470
L-f	P	2071	1608	1812	3348	1802	2714	2958	3128	1280	5616	--
	L	8720	11658	13288	9052	8798	9086	14442	10472	5120	15984	--
	M	109	134	0	0	0	0	0	0	0	216	--

o = no marked ear
R = right ear marked
L = left ear marked

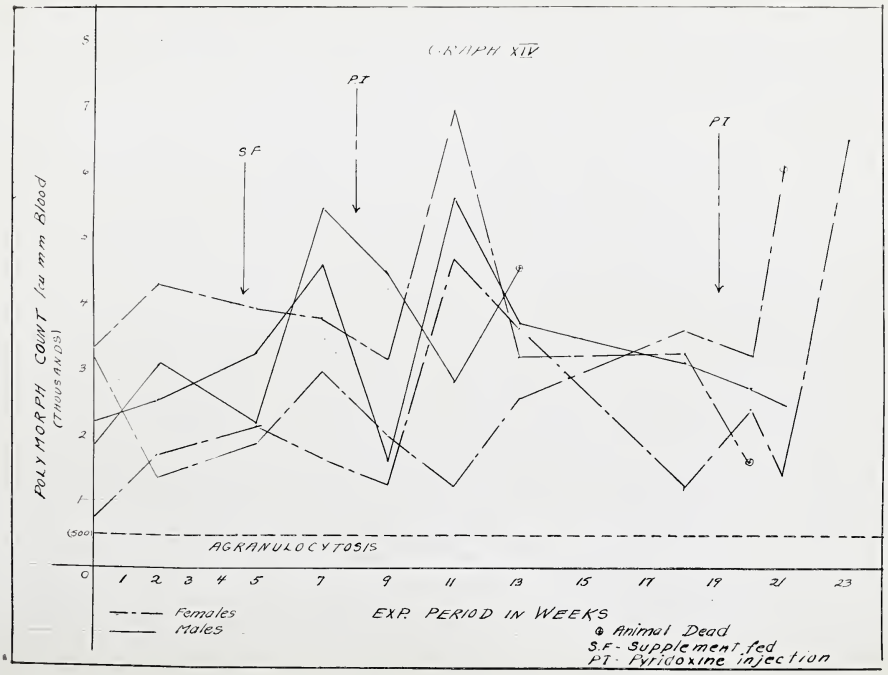
m = male
f = female

P = Polymorphs
L = Lymphocytes
M = Monocytes

Graph XIII. The effect of pyridoxine on leukopenia induced by .25% pyramidon in a B-complex free diet (Albino rats, 7 weeks age).



Graph XIV. The effect of pyridoxine on a low polymorph count induced by .25% pyramidon in a B-complex free diet.



Pyridoxine + 2% Sulfaguanidine.

Table XV. Total leukocyte count per cubic millimetre blood

Rat	Weeks after experiment began								
	0	2	4	6	9	11	14	15	16
o-m	9800	13000	10600	14800	7350	10150	3800	5400	--
o-f	10600	--	--	--	--	--	--	--	--
R-m	8500	14500	9750	--	--	--	--	--	--
R-f	8250	--	--	--	--	--	--	--	--
L-m	6400	20350	9700	--	--	--	--	--	--
L-f	8400	20100	13500	15300	10050	30650	--	--	--

m = male
f = female

o = no marked ear
R = right ear marked
L = left ear marked

Pyridoxine + 2% Sulfaguanidine.

Table XVI. Differential count per cubic millimetre blood

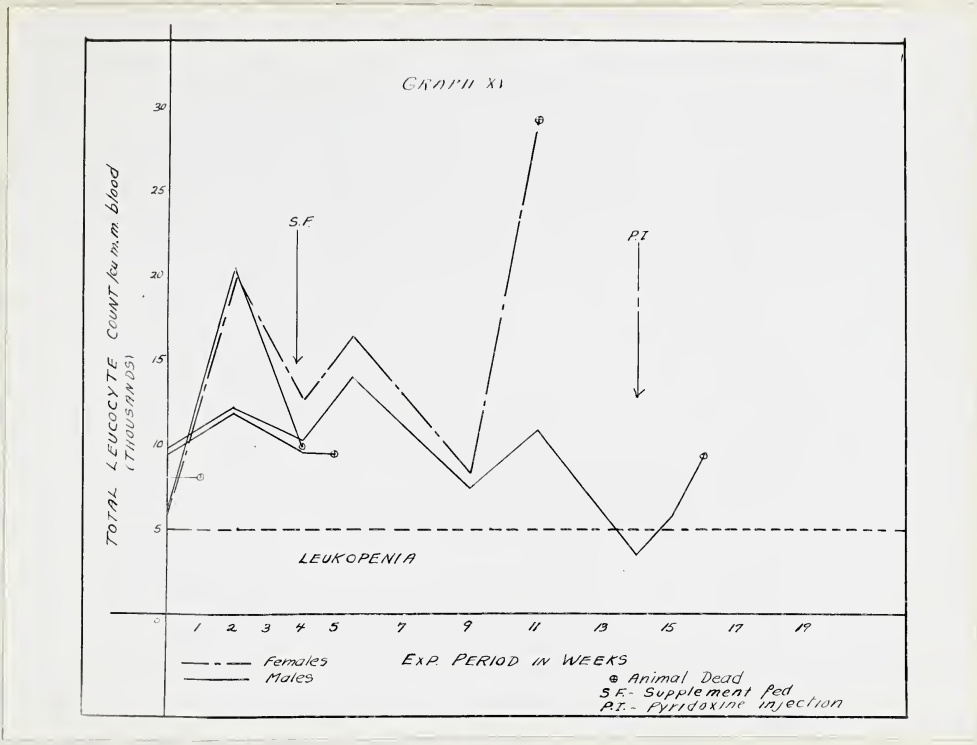
Rat		Weeks after experiment began								
		0	2	4	6	9	11	14	15	16
o-m	P	1274	1820	1378	1628	2774	2222	380	810	--
	L	8526	11180	9222	13172	4526	7878	3420	4590	--
	M	0	0	0	0	0	0	0	0	--
o-f	P	1484	--	--	--	--	--	--	--	--
	L	9010	--	--	--	--	--	--	--	--
	M	106	--	--	--	--	--	--	--	--
R-m	P	1275	2900	1940	--	--	--	--	--	--
	L	7225	11600	7663	--	--	--	--	--	--
	M	0	0	97	--	--	--	--	--	--
R-f	P	738	--	--	--	--	--	--	--	--
	L	6642	--	--	--	--	--	--	--	--
	M	0	--	--	--	--	--	--	--	--
L-m	P	832	2842	1358	--	--	--	--	--	--
	L	5568	17458	8342	--	--	--	--	--	--
	M	0	0	0	--	--	--	--	--	--
L-f	P	924	6834	1755	4284	4800	15300	--	--	--
	L	7476	13266	11745	11016	5200	14688	--	--	--
	M	0	0	0	0	0	612	--	--	--

o = no marked ear
R = right ear marked
L = left ear marked

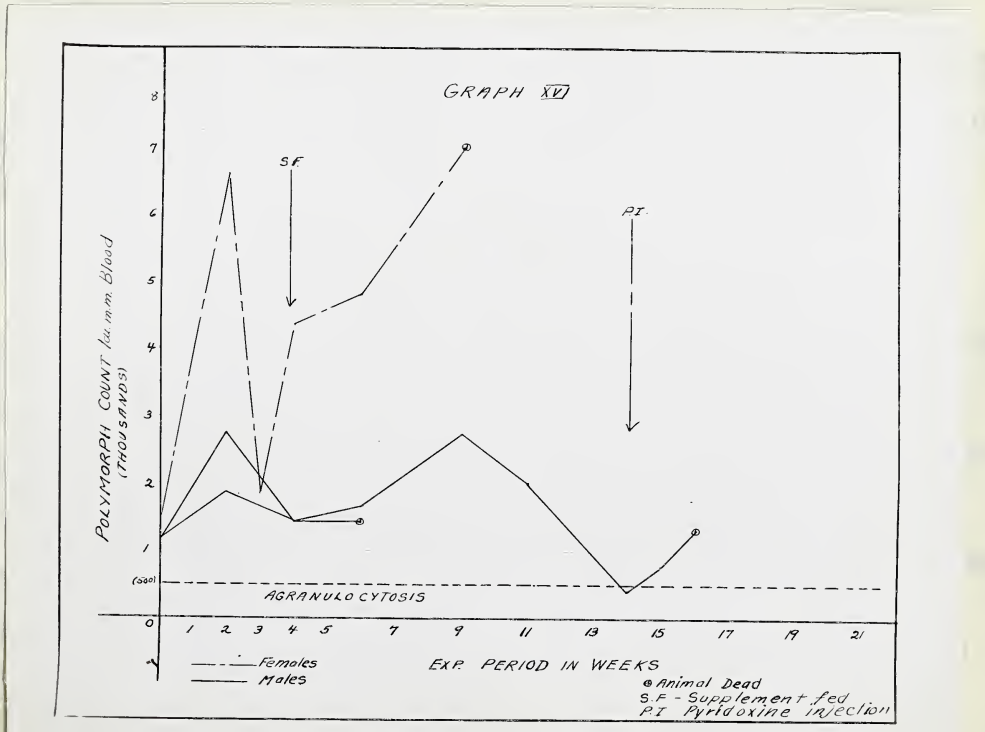
m = male
f = female

P = Polymorphs
L = Lymphocytes
M = Monocytes

Graph XV. The effect of pyridoxine on leukopenia induced by 2% sulfaguanidine in a B-complex free diet (Albino rats, 6 weeks age).



Graph XVI. The effect of pyridoxine on a low polymorph count induced by 2% sulfaguanidine in a B-complex free diet.



DISCUSSION

From Table and Graph VII there is evidence of a significant rise in total leukocytes after the injection of pyridoxine. Since pyridoxine was the only deficiency the uniform rise in leukocytes following the injection could be attributed to pyridoxine. Agranulocytosis was not produced (Graph VIII). After giving pyridoxine, the polymorph trend was not significantly different from previous results to justify the conclusion that pyridoxine was responsible. At the same time, a slight rise did occur.

(Tables and Graphs IX and X). Neither leukopenia nor agranulocytosis occurred in these animals. After pyridoxine injection the leukocyte counts rose to a higher level. Although the rise in response to pyridoxine was irregular, the effect in all animals on the absolute count was a significant increase.

Too few animals survived in Group 7 (Tables and Graphs XI and XII) to permit any general conclusions. It seems apparent that pyramidon in the concentrations fed produced earlier fatality than did gardan.

The value of the experiment in relation to pyridoxine effect is diminished by the number of survivors. Of significance is the response of one animal to repeated injections of pyridoxine. Both the total leukocyte and polymorph counts rose in close correlation to each other after each injection. It is reasonable to conclude that pyridoxine was responsible for the rise in counts in this animal.

In Tables and Graphs XIII and XIV, the total leukocyte counts responded with a uniform rise after injection then fell. Since the counts were relatively low and later a rise in the majority of cases occurred, the pyridoxine must have been responsible. Following the injection of two females, their total leukocyte and polymorph counts rose in correlation. This showed significant evidence in suggesting that pyridoxine was the responsible factor. The polymorph count of most animals was at its peak after pyridoxine injection. The response in general was a rise in count but this was not significant enough from which to draw conclusions. Agranulocytosis was not produced. The pyramidon did not interfere with leukopoiesis sufficiently enough to produce this condition.

The number of survivors shown by Tables and Graphs XV and XVI seriously limits the value of the experiment with respect to the response to pyridoxine. The lone survivor showed a favorable response to pyridoxine: a close correlation occurred between the rise in leukocytes and the rise in polymorphs. The unusual response of one animal (female) leads one to suspect unusual sensitivity to sulfaguanidine.

Animals showed symptoms similar to those occurring in Part I (coarse hair, loss of hair, loss of weight, and toward death a cramped condition with faint body tremors). Rats fed 2 per cent gardan and pyramidon rapidly began passing blood in both faeces and urine. $NaHCO_3$ was given and the quantity of both drugs reduced to .25 per cent.

SUMMARY

The response of rats with low leukocyte and polymorph counts (induced by feeding .25 per cent gardan, .25 per cent pyramidon and 2 per cent sulfaguanidine) to pyridoxine, injected subcutaneously, was:

(1) In general a significant rise in both total leukocyte and polymorph counts;

(2) In nearly all instances of leukopenia and agranulocytosis rises in counts occurred to correct these conditions;

(3) In several isolated cases after repeated injections of pyridoxine, a close correlation occurred between the rise in total leukocytes and polymorphs.

PART III

"The effect of the toxin of *stachybotrys alternans* (a saprophytic mold) on Piebald rats."

INTRODUCTION

A review by V. G. Drobotko (28) brought to our attention the presence of two new diseases, stachybotrytoxicosis of horses and stachybotrytoxicosis of humans. Both were discovered in the Soviet Ukraine and up to now there have been no reports elsewhere of their occurrence. Leukopenia and agranulocytosis are characteristic of the late stages of these diseases.

The source of the causative agent in each case was traced to a saprophytic mold, stachybotrans alternans, found in moist hay.

In horses the disease manifested itself in three clinical stages:

(1) An irritation of lips, mouth, throat and nose.

(2) Leukopenia gradually appeared in addition to signs in (1).

(3) A rise in temperature maintained until death. Leukopenia persists, necrotic ulcers developed and agranulocytosis developed. The majority of cases recovered after the first or second stage.

Humans who came into contact with the infected hay contracted a moist dermatitis, anginal pharyngitis with a bloody exudate and cough. Pains in the throat, a burning in the nose and chest congestion were common symptoms. Only in a few cases did a decrease in the leukocyte count occur, but never was it below 2000 cells per cubic millimetre of blood.

The mold was grown on agar plates. Ether extracts of the mold injected into horses proved the presence of an etiologic agent ("Toxin") capable of causing the disease. All observations indicated a definite chemical substance which poisons the organism; a bacteriologic factor may play some part.

It is logical to assume that the severe degree of leukopenia in horses and mild leukopenia in humans may be attributed to the different methods of acquiring the toxin. Horses obtained large amounts of toxin orally (eating hay) with the resultant relatively rapid absorption and high concentration in the blood stream. With humans, only through the respiratory tract or skin (slow methods of absorption) did the toxin gain admission to the blood.

PLAN OF EXPERIMENT

To show the effect of the toxin on Piebald rats.

Animals

Six Piebald rats (Departmental Colony) were divided into two groups consisting of three females and three males. Each group was placed in a separate cage. Water and a complete stock diet were allowed ad lib.

Blood counts were taken immediately and on successive days as indicated in the graphs following.

The males were subcutaneously injected with .1 cubic centimetre of toxin. Females were orally fed by pipette a like amount.

Counting

Total and differential white cell counts were made as in Part I.

Preparation of Toxin

Nine cultures of stachybotrys alternans were made in petri dishes (Agar medium) and 24 liquid cultures. The 33 molds were ether extracted four times, filtered and the ether evaporated. The oil and solid material resulting (1.5 grams) was made up to 9 cubic centimetres with sesame oil. This solution, containing the toxin, was used in the experiment.

RESULTS

(1) Shown by the total cell count in Table XVII and Graph XVII, and the polymorph count in Table XVIII and Graph XVIII.

(2) Symptoms shown by the animals are given in the discussion.

DISCUSSION

The sudden rise in total leukocyte count above normal values can be attributed to the toxin. Individual animals showed a greater resistance to the toxin than others (death of two). Although a small number of animals was used, it seems logical to assume that oral administration caused death more rapidly than subcutaneous administration.

The oral fed rats showed similar symptoms to those of horses which had been feeding on hay infected with the toxin.

Swollen, purplish tongues, blood and mucus excretion through the nostrils, respiration trouble and body tremors were characteristic symptoms shown by the orally fed rats. Injected rats showed a bunched intoxicated condition as the experiment continued. Body lesions at the site of injection became badly inflamed, failed to heal and caused such discomfort to the animals that further injections were halted. These animals regained normal health later.

Agranulocytosis did not appear in the experiment. Time might be a factor, since leukopenia and agranulocytosis in horses (28) did not appear until six weeks to a month after the initial absorption of the toxin. This sudden rise in leukocyte count with the rats runs parallel to the picture with horses.

If continued over a long period, the animals may have shown the second stages characterized by leukopenia. In rats this remains to be seen.

SUMMARY

The toxin of stachybotrys alternans was administered to Piebald rats:

(1) Death occurred in some cases.

(2) Rats fed orally showed the first symptoms typical of stachybotrytoxicosis of horses.

(3) Leukopenia or agranulocytosis was not produced.

Toxin of S. A.

Table XVII. Total leukocyte count per cubic millimetre blood

Rat	Experimental period (days)			
	0	5	7	11
o-m	9400	6800	22000	22000
o-f	8450	6200	16400	--
R-m	8750	20400	19500	21500
R-f	8350	23000	17000	17200
L-m	8250	--	--	--
L-f	6400	--	--	--

m = male
f = female

o = no marked ear
R = right ear marked
L = left ear marked

Table XVIII. Differential count per cubic millimetre blood

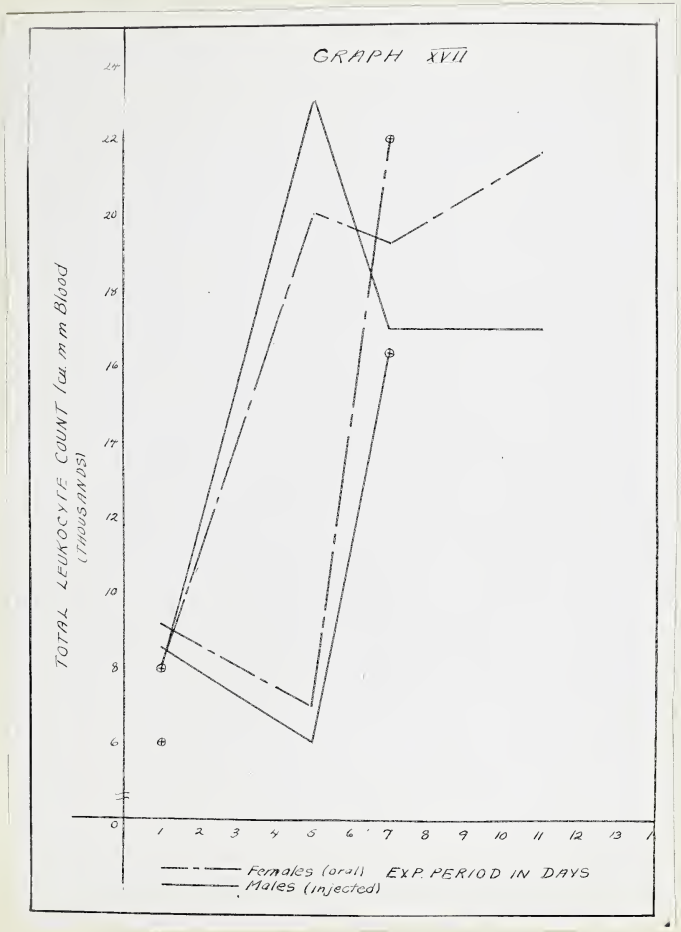
Rat		Experimental period (days)			
		0	5	7	11
o-m	P	2726	1020	6160	3300
	L	6392	5712	15840	18700
	M	94	68	0	0
o-f	P	2268	866	4920	--
	L	6132	4132	11316	--
	M	0	0	164	--
R-m	P	2349	3264	7605	3010
	L	6003	16932	11895	18275
	M	0	204	0	0
R-f	P	1162	4140	6460	4472
	L	7058	18860	10370	12556
	M	83	0	170	172
L-m	P	1886	--	--	--
	L	5968	--	--	--
	M	0	--	--	--
L-f	P	768	--	--	--
	L	5440	--	--	--
	M	0	--	--	--

o = no marked ear
R = right ear marked
L = left ear marked

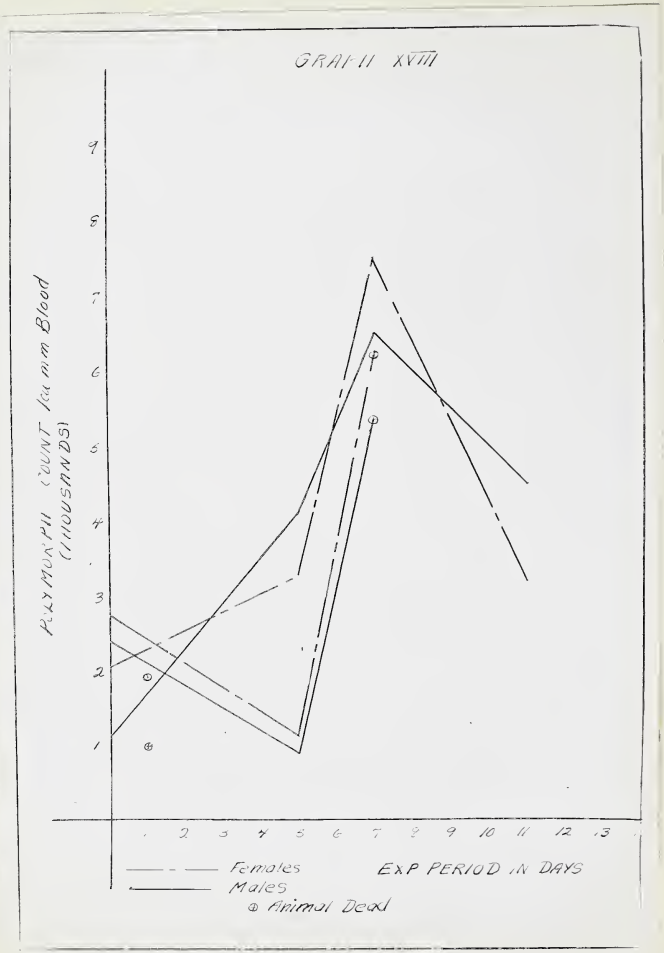
m = male
f = female

P = Polymorphs
L = Lymphocytes
M = Monocytes

Graph XVII. The effect of the toxin of S. A. on the total leukocyte count.



Graph XVIII. The effect of the toxin of S. A. on the granulocytic count.



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APPENDIX

APPENDIX

PREPARATIONS AND METHODS OF FEEDING

1. The pyridoxine free, B-complex supplement was prepared as follows:

Stock solutions

Thiamin solution	-	1 gm. in 20 c.c. water
Riboflavin susp.	-	1 gm. in 20 c.c. Biotin
Ca Pantothenate solution	-	1 gm. in 20 c.c. water
Niacin solution	-	1 gm. in 40 c.c. water
Choline HCl solution	-	20 gm. in 40 c.c. water
Inositol solution	-	1 gm. in 20 c.c. water
Para. Am. Benz. Acid solution	-	1 gm. in 20 c.c. EtOH (95%)

Supplement prepared from stock solutions:

Thiamin	1	c.c.
Riboflavin	in 20	c.c. Biotin cone
Ca Panto.	2	c.c.
Niacin	10	c.c.
Choline	10	c.c.
Inositol	5	c.c.
Para. A. B. Acid	2	c.c.

2. Oral feeding was accomplished by placing the end of the pipette into the rat's mouth.

3. Pyridoxine HCl solution:

50 gms. of pyridoxine HCl dissolved in 100 c.c of water.

.5 c.c. of solution = 50 mgms. pyridoxine HCl.

4. Each subcutaneous injection was (.5 c.c. of solution) given in the region of the lower thigh.

5. Drugs used were received gratis from the following manufacturers:

Sulfaguanidine	- John Wyeth and Bros. Limited
Gardan	- Winthrop Chemical Co. Ltd.
Pyramidon	- Winthrop Chemical Co. Ltd.

Thiouracil	- John Wyeth and Bros. Limited
Nicotinic Acid	- John Wyeth and Bros. Limited
P. Amino B. Acid	- John Wyeth and Bros. Limited
Riboflavin	- John Wyeth and Bros. Limited
Biotin	- John Wyeth and Bros. Limited
Pyridoxine HCl	- John Wyeth and Bros. Limited.

6. Double distilled water (glass still). All solutions were preserved at refrigeration temperature in tightly stoppered brown glass bottles.

SECTION B

ABSORPTION AND STORAGE OF CAROTENE (PROVITAMIN A) IN THE RAT.

INTRODUCTION

ABSORPTION AND STORAGE OF CAROTENE (PROVITAMIN A) IN THE RAT.

INTRODUCTION

Vitamin A occurs only in the animal organism. The compounds found in plants with vitamin A activity for animals belong to the class of carotenoids containing 40 carbon atoms. They are converted in the animal body into other substances having vitamin A activity. Rats are usually used for provitamin studies and it is assumed that compounds of provitamin activity for rats also exhibit this activity for man (1).

The efficiency with which provitamin A is converted to vitamin A varies in different animals, rats being most efficient. Cats are not capable of this conversion (3, 7).

B-carotene administered orally to the Albino rat is absorbed from the gastro-intestinal tract, converted to vitamin A and stored chiefly in the liver. Small amounts are stored in the corpus luteum, adrenal cortex, in the retina as visual purple, renal cortex and tissue fat. Traces occur in the lungs and the pars intermedia of the pituitary (6). Carotene is found in the liver, dentin of teeth and the testes.

The absorption of B-carotene and its conversion depend upon a mechanism yet unknown. Most evidence points to the fact that conversion of B-carotene to vitamin A takes place in the Kupffer cells of the liver, that an enzymatic process is involved.

There is also evidence that cells of the intestinal villi may take part in the conversion (1, 16, 21).

A critical review of the literature reveals a wide diversity of experimental conditions among the various investigators: differences in animals (sex, age, stage of liver, depletion of vitamin A), in diets (purity, nature), in carotene dosages used, and in the duration of experimental periods. The previous dietary history has a marked effect on the rate of absorption and degree of vitamin A storage. Adequately fed animals store vitamin A more efficiently than vitamin A deficient animals (7, 14, 21). Sereda (39) observed that the rate of absorption of vitamin A was increased by preliminary priming doses of vitamin A. When animals were fed a vitamin A free diet for more than one week and subsequently provided with carotene, irregularity in vitamin A storage is attributed to irregular intestinal absorption (17).

It is generally agreed that vitamin A is utilized more efficiently than B-carotene. Clayton and Baumann (9) attributed this to the fact that vitamin A is an alcohol and B-carotene a hydrocarbon. Russell (10), Popper (16) and Kemmerer et al (20) observed that the absorption of vitamin A and B-carotene is proportional to the dose fed. With vitamin A this is true up to a maximum of 300,000 I. U. Using daily doses of 5,000 I. U. in vitamin A depleted animals, Sereda has shown that complete "liver saturation" could be affected in about 20 days (100,000 I. U.), following which further increase could not be produced.

Experimental studies have revealed a correlation between

the rate of carotene and lipid absorption. Thorbjarnarson and Drummond (15), Wilson et al. (19), Shaw et al. (21) and Irvin et al. (18) found that significant quantities of carotene are absorbed when fed with gall bladde bile and pancreatic lipase. This could not be confirmed by Dzialoszynski et al. (13) and Goss and Guilbert (26).

The utilization of carotene by rats is influenced by the nature of the oil used as diluent (19, 22). Wesson oil (20) and cod liver oil (29) provided more efficient utilization of B-carotene than cottonseed oil. Slanetz and Scharf (24) observed that mineral oil interferes with full utilization.

A report by Guggenheim and Koch (12) suggests a protective action of vitamin E in the small intestine enhancing vitamin A absorption and storage. Russell (10) postulates a similar action of phosphatides.

Frazer, Schulmann and Stewart (8) showed that the rate of fat absorption was increased when emulsifying agents were used.

Evidence put forward by this paper caused a similar emulsifying system to be used in the following experiments.

PART I

"Comparison of the efficiency of propylene glycol and cottonseed oil as B-carotene solvents on the storage of B-carotene and vitamin A in the liver of Albino rats."

PLAN OF EXPERIMENT

B-carotene in cottonseed oil and in propylene glycol was fed to albino rats. The storage of B-carotene as vitamin A in the liver was measured.

Animals

Adult female albino rats (Departmental Colony) were fed a vitamin A free diet when their young were born. Young females at weaning (3 weeks) were fed the same diet and allowed water ad lib.).

Two groups of animals (7 weeks of age) were used in each experiment. Each group of 20 was placed in a separate cage and fed the vitamin A free diet. They were allowed water ad lib. One group was fed a daily supplement of 1,000 I. U. of B-carotene in cottonseed oil; the other group received 1,000 I. U. of B-carotene in propylene glycol.

One animal out of each group was killed with ether every four to five days. The drained livers were weighed and a chemical analysis performed to determine vitamin A and B-carotene content.

Analysis

The Carr Price procedure (1) was followed and modified (2) for this purpose.

Flow Sheet

1. Minced organs + 40 c.c. NaOH (5%) - heated at 60°C. for one hour - shaken occasionally.

2. Solution cooled (room temperature) and measured in

graduate cylinder.

3. Duplicate 10 c.c. aliquots + 4 c.c. KOH (60%) in H_2O + 10 c.c. EtOH (95%), refluxed 5 minutes at temperature of boiling H_2O.

4. Flasks cooled - room temperature.

5. Extraction. Three times - 10 c.c. of petroleum ether (B. P. under 40° C.).

6. Washing. Once with 15 c.c. of double distilled H_2O.

7. Drying. Over anhydrous Na_2SO_4 for at least 4 hours.

8. Solution filtered and Na_2SO_4 washed thoroughly with petroleum ether (B. P. under 40° C.).

9. Filtrate volume measured in graduate cylinder.

10. Ten c.c. sample placed in cuvette tube: comparator tube 10 c.c of petroleum ether (B. P. under 40° C.).

(i) Coleman Bell Double Spectrophotometer used with selective filter at 440 mu. Transmission read directly and the quantity of carotene obtained by reference to curve obtained by treating a solution of known B-carotene content in a manner similar to the procedure outlined.

(ii) Sample replaced in original cylinder, petroleum ether evaporated under nitrogen stream at 30° to 40° C. and the residue dissolved in 10 c.c. of chloroform. Vitamin A determined by method of Carr and Price using the color reaction with antimony trichloride and selective filter at 620 mu. Results were calculated using the equation:

$$(\log. 100 - \log. \text{reading}) \times .0525 \times 58500 \times \frac{\text{c.c's. solution}}{100}$$

This for 10 c.c. aliquot of solution. Ten c.c. chloroform used. Results expressed in I. U.

Standard B-carotene Curve used as reference.

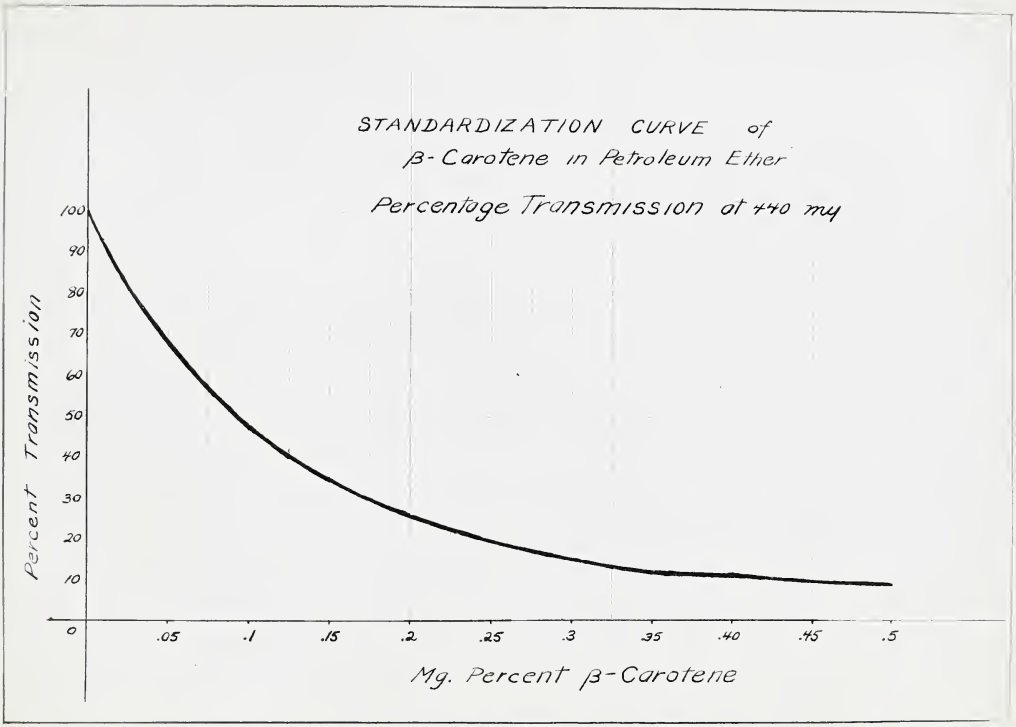


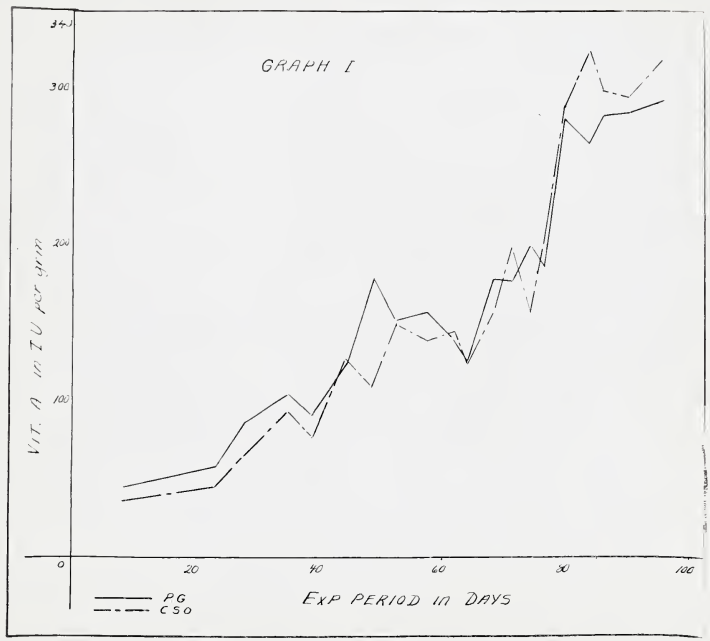
Table I. Storage of vitamin A and B-carotene in liver tissues.

Time in days	C. S. O. Vitamin A	C. S. O. B-carotene	P. G. Vitamin A	P. G. B-carotene
0	.85	0	1.20	0
11	37.70	6.80	46.20	9.70
23	42.40	6.97	55.20	7.00
28	64.20	6.01	85.30	9.60
35	93.40	12.20	104.60	9.10
38	78.70	5.90	87.70	6.05
42	128.70	15.10	122.40	7.10
46	110.30	7.49	178.50	13.90
50	147.40	8.44	148.10	7.99
55	137.10	9.54	156.60	8.60
59	144.80	8.92	142.40	14.10
63	121.00	5.50	123.10	7.20
67	156.10	6.25	176.20	5.75
71	195.10	10.30	175.70	17.60
74	155.30	7.60	200.20	7.74
77	198.10	7.96	186.20	5.94
80	275.60	16.40	263.30	8.21
84	303.10	7.83	244.30	9.20
88	280.90	7.97	265.60	7.41
92	277.70	10.30	272.80	7.80
97	298.70	8.40	275.60	7.52

C. S. O. = Cottonseed Oil.
P. G. = Propylene Glycol.

Vitamin A and B-carotene as International Units per gram of liver tissue.

Graph I. Comparison of Vitamin A storage in the liver, using cottonseed oil and propylene glycol as B-carotene diluents.



RESULTS

1. Table I and Graph I summarize the results obtained per gram of liver tissue.

DISCUSSION

Table I and Graph I illustrate that there is no significant difference in the rate and extent of vitamin A storage using either cottonseed oil or propylene glycol. The two curves parallel each other closely throughout the experiment. Since the animals were chosen of about equal weight at the time of liver analysis, any minor degree of storage rates could be attributed to individual animal differences in storage efficiency rather than to the difference in the two solutions used.

The extent of B-carotene storage tended to remain static after the initial storage. This suggests that the liver has only a limited capacity for storing B-carotene and amounts in excess of this are either converted to vitamin A or excreted.

SUMMARY

1. No difference was observed in the efficiency of liver storage of vitamin A by the albino rats, when B-carotene was fed in cottonseed oil or propylene glycol.

PART II

"The effect of lipids on beta carotene absorption and storage of vitamin A in the albino rat."

PLAN OF EXPERIMENT

The absorption of B-carotene and its conversion to vitamin A depends on factors the nature and mechanism of which are as yet uncertain. Experimental studies suggest a correlation between the rate of B-carotene and lipid absorption (5, 6, 7). These studies were carried out on animals in various stages of depletion and it was thought that greater uniformity in results might be obtained in completely depleted animals.

Animals

Animals were prepared and handled in the manner already described (part I).

Three groups of animals seven weeks of age were used. Each group of 24 was placed in a separate cage and fed a vitamin A free diet. They were allowed water ad lib. Two animals from each group were fed the test dose of carotene. At varying time intervals both animals were killed with ether and their digestive tracts assayed for B-carotene content. Vitamin A assays were performed only on the livers.

RESULTS

The results are presented in Tables II, III, IV and V.

(1) Table II. After feeding 2100 I. U. of B-carotene in cottonseed oil (conc.).

(2) Table III. After feeding 1400 I. U. of B-carotene (conc.) with sodium taurocholate.

Table II. B-carotene in cottonseed oil expressed as per cent of the dose

Time in hours	Stom. + Cts.	S.I. + Cts.	L.I.+ Cts. + Caecum Cts.	Liver Vit.A	Liver B-C.	Total
0	0	0	0	1.14	0.22	
1	52.50	5.4	0.3	1.39	0.56	60.1
2	57.20	24.5	0.9	1.17	1.20	84.9
3	20.90	28.4	2.6	1.20	1.20	54.3
4	22.30	9.2	2.9	1.40	1.20	32.0
5	18.20	11.8	5.3	2.30	1.70	39.3
6	15.30	7.8	18.4	2.70	1.60	45.8
7	17.60	10.5	27.6	2.90	1.60	60.2
8½	14.30	6.8	40.8	3.30	2.70	67.9
12	2.48	4.2	38.4	3.82	1.60	50.5
24	1.35	1.9	10.6	4.10	1.50	19.4

Stom. = Stomach
S.I. = Small Intestine
L.I. = Large Intestine
Cts. = Contents
B-C = Beta Carotene

Table III. Absorption of B-carotene plus bile salts. B-carotene expressed as per cent of the dose.

Time in hours	Stom. + Cts.	S.I. + Cts.	L.I. + Cts.	Liver Vit.A	Liver B-C.	Total
0	0	0	0	.89	.56	
1	48.4	13.20	0.60	.87	1.30	64.4
2	51.4	24.10	1.73	.91	1.95	80.1
3	25.1	32.40	3.20	1.15	1.85	63.7
4	21.1	11.20	3.55	1.68	2.84	40.4
5	14.3	4.80	5.90	1.82	2.87	29.7
6	12.3	12.80	21.00	2.10	2.56	50.7
7	15.2	9.50	21.30	2.55	3.88	52.4
8½	8.2	6.51	47.80	2.70	4.18	69.4
12	5.5	1.31	46.60	5.64	3.38	62.4
24	2.8	1.05	9.30	6.31	2.65	22.2

Stom. = Stomach
S.I. = Small Intestine
L.I. = Large Intestine
Cts. = Contents
B-C. = Beta carotene

Table IV. Absorption of B-carotene plus an emulsifier. B-carotene expressed as per cent of the dose.

Time in hours	Stom. + Cts.	S.I. + Cts.	L.I. + Cts.	Liver Vit.A	Liver B-C.	Total
0	0	0	0	1.27	.38	
1	40.80	24.10	.95	1.50	1.40	68.7
2	47.30	24.70	1.50	1.57	1.46	76.5
3	29.30	33.30	2.10	1.90	1.50	68.1
4	20.50	45.80	4.60	2.23	1.61	74.7
5	7.40	12.50	25.60	2.24	1.74	49.5
6	6.50	5.70	24.70	2.34	1.83	41.1
7	5.70	7.80	46.00	2.88	2.11	64.5
8½	6.80	7.20	38.40	3.50	2.31	57.9
12	2.49	2.50	13.20	3.80	2.73	23.6
24	1.00	1.32	2.20	5.70	3.34	13.5

Stom. = Stomach
S.I. = Small Intestine
L.I. = Large Intestine
Cts. = Contents
B-C. = Beta Carotene

Table V. B-carotene remaining in gastro-intestinal tract expressed as per cent of the dose.

Time in hours	C. S. O. in G.I.	C. S. O. Assume Absorp.	B. S. in G.I.	B. S. Assume Absorp.	Emuls. in G.I.	Emuls. Assume Absorp.
0	--	--	--	--	--	--
1	58.2	--	54.2	--	65.8	--
2	82.6	17.4	77.2	22.8	73.5	26.5
3	51.9	48.1	60.7	39.3	64.7	35.3
4	34.4	65.6	35.8	64.2	70.9	29.1
5	35.3	64.7	25.0	75.0	47.8	52.2
6	41.5	58.5	46.2	53.8	36.9	63.1
7	55.7	44.3	46.0	54.0	59.5	40.5
8½	61.9	38.1	62.5	37.5	52.4	47.6
12	45.1	54.9	53.4	46.6	18.2	81.8
24	13.8	86.2	13.1	36.9	4.5	95.5

C.S.O. = B-Carotene in cottonseed oil
B.S. = B-Carotene in bile salts
Emuls. = B-Carotene in emulsifier
G.I. = Gastro-intestinal tract

(3) Table IV. After feeding 1300 I. U. of B-carotene (conc.) with an emulsifier.

Details of the preparations are described in the appendix.

DISCUSSION

From Tables II, III and IV it can be seen that there is no great difference between the rate of B-carotene absorption using bile salts and the emulsifying agent.

Absorption of B-carotene takes place solely in the small intestine. By totalling the first three columns in each of the Tables II, III and IV, the percentage of the dose of B-carotene remaining in the gastro-intestinal tract is obtained (Table V). If it is assumed that there is no destruction of carotene, the remainder of the dose represents carotene absorbed into the blood. Any large differences in the percentage of the dose found in the liver must represent destruction in the gastro-intestinal tract.

In general, the rates of absorption of B-carotene in cottonseed oil and B-carotene with bile salts parallel each other. The rate and extent of absorption with the emulsifying agent was less than in the other two groups. Greatest absorption took place in the five hour group using bile salts.

The rate of storage of vitamin A in the liver was approximately equal in all groups. The extent of storage after the 24 hour interval was significantly greater in the bile salt group. The emulsifying group and cottonseed oil group show little difference in this respect.

SUMMARY

After feeding albino rats dosages of 2100 I. U. of B-carotene, 1400 I. U. of B-carotene with a bile salt, and 1300 I. U. of B-carotene with an emulsifying agent, it was concluded that:

(1) Bile salts slightly improved the rate of absorption of B-carotene from the gastro-intestinal tract. Increased liver storage is possibly due to the increased amount of carotene absorbed.

(2) No improvement of B-carotene absorption and vitamin A storage occurred with the emulsifying agent.

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APPENDIX

APPENDIX

PREPARATIONS AND METHODS OF FEEDING

Part I

1. Stock B-carotene in cottonseed oil.

(1) Cottonseed oil was added so that

.1 c.c. solution = 1000 I. U. of B-carotene.

(2) Propylene glycol added so that

.1 c.c. solution = 1000 I. U. of B-carotene.

Solutions were kept in refrigerator in brown glass bottles.

2. Oral feeding was accomplished by placing the end of a graduated pipette into the rat's mouth.

3. Reagents:

(1) 95% ethanol (C. P.).

(2) Low B. P. petroleum ether, C. P. Petroleum ether B. P. below 60° C., was redistilled using a fractionating column and the fraction boiling under 40° C. used.

(3) 60% KOH in water.

(4) Chloroform (C. P.). Exposure to air (H_2O vapor) kept at a minimum by opening small quantities for use at any one time.

(5) Anhydrous sodium sulfate (C. P.).

(6) Antimony Trichloride in chloroform (25% W/V).

(7) Double distilled water (glass still).

Part II

1. Stock B-carotene in cottonseed oil.

(1) .15 c.c. solution = 2100 I. U. of B-carotene.

(2) Sodium taurocholate was measued as 25 mgms. per .15 c.c. of stock solution.

.15 c.c. of solution = 1400 I. U. of B-carotene + Na taurocholate.

(3) The emulsifier was measured as 25 mgms. per .15 c.c. of stock solution.

.15 c.c. of solution = 1300 I. U. of B-carotene + emulsifier.

Emulsifier:

3% Na Taurocholate
36% Lecithin
61% Oleic acid.

Estimations of B-carotene were performed on .15 c.c. of each solution, measured from the feeding pipette.

2. Oral feeding was accomplished by placing the end of the pipette into the rat's mouth.

3. Compounds:

(1) B-carotene - "Smaco."

(2) Sodium Taurocholate - "Eimer and Amend."

(3) Propylene Glycol - "Eastman Kodak Co."

(4) Oleic Acid (C. P. free from linoleic acid) - "Eimer and Amend."

(5) Cottonseed Oil - "Fisher Scientific Co."

(6) Lecithin - "Pfanstiel."